

Tobacco Mosaic Virus Inoculum Increase in Tobacco Infected at Flower Removal

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ABSTRACT

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The transmission of tobacco mosaic virus (TMV) during flower removal (topping) in tobacco and the subsequent translocation of the virus to the roots of infected plants was demonstrated using ELISA and plant bioassay techniques. The use of axillary bud (sucker) inhibitors (long-chain aliphatic alcohols and maleic hydrazide) obscured the symptoms of TMV infection in plants inoculated during topping. Infection of tobacco roots after transmission of TMV at topping provides a potential for tremendous proliferation of inoculum sources for the next year's crop.

Tobacco mosaic virus (TMV) consistently ranks among the most costly

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tobacco diseases in the flue-cured tobacco-producing region of the southeastern United States (3,8). Comprehensive, integrated TMV control programs have been strongly advocated in most states that produce flue-cured tobacco (3, 8-10); nevertheless, losses to TMV remain high. TMV is difficult to control because of the exceptional ease by which it is mechanically transmitted.

Most TMV control programs advocate sanitation in the plant bed and during transplanting, prevention of mechanical

transmission during cultivation, timely destruction of crop debris to reduce overwintering inoculum, and crop rotation. Little or no mention is made of the potential for widespread transmission and proliferation of inoculum sources of TMV during topping (flower removal), when each plant is handled manually. Because a primary overwintering source of TMV is root debris from infected tobacco plants (6), the potential for the production of large numbers of inoculum sources is great if virus transmission during topping leads to subsequent translocation to and infection of tobacco roots. This potential has never been evaluated, probably because it has been shown that inoculation of tobacco with TMV at topping has no apparent effect on yield, quality, plant height, or chemical composition of the inoculated crop (2). Thus, a lack of concern for late-season spread of TMV in the immediate crop has discouraged investigation into

the role of late-season inoculum buildup for subsequent crops.

The purpose of this study was to determine if TMV transmission during topping resulted in translocation to and infection of the roots of tobacco plants, which would serve as potential inoculum sources for a subsequent crop. The effect of chemical sucker (axillary bud) control on this process was also determined.

MATERIALS AND METHODS

Tobacco (*Nicotiana tabacum* L. 'Clemson PD-4') plants were grown at the Pee Dee Research and Education Center at Florence, SC, using flue-cured tobacco production practices recommended for South Carolina (3). Each plot consisted of a single row of 30 plants spaced 50 cm apart within the row. The plots were 122 cm apart, replicated three times in a randomized complete block design.

Treatments consisted of 1) TMV inoculation at topping plus chemical sucker control, 2) TMV inoculation at topping with no sucker control, 3) no TMV inoculation at topping plus chemical sucker control, and 4) no TMV inoculation at topping and no sucker control. Chemical sucker control consisted of two applications of long-chain aliphatic alcohols (Off-Shoot T) at 5.5 kg a.i./ha in 445 L of water on 24 and 28 June, beginning when 50% of the plants were in the elongated button stage of flowering, followed by an application of maleic hydrazide (Royal MH-30) at 2.5 kg a.i./ha in 470 L of water on 5 July. Manual breaking off of the flower stalks (topping) was done on 28 June. Treatments not receiving TMV inoculation were topped first. Hands were contaminated with TMV by rubbing them with ground, TMV-infected tobacco leaves before topping each of the remaining treatments.

The plots were observed for TMV symptoms on 28 June, immediately before topping, and again on 31 July, when a root sample was collected from each of 10 randomly chosen plants per plot. Pruning shears and digging tools were disinfested by spraying with a 2.6% solution of sodium hypochlorite after each plot was sampled. Root samples were washed and frozen until assayed for TMV. A motorized "leaf squeezer" (8) was used to grind 0.5 g of root tissue in 2 ml of 0.03 M sodium phosphate buffer, pH 7.0, with 0.05% Tween 20 and 0.02 M sodium diethyldithiocarbamate for enzyme-linked immunosorbent assay (ELISA) and plant bioassay. The ELISA protocol was as described previously (7), with immunoglobulin at 10 µg/ml for coating plates and alkaline phosphatase-conjugated immunoglobulin at 2.5 µg/ml. The plant bioassay involved rubbing corundum-dusted (600-mesh) leaves of 5-wk-old, TMV-hypersensitive tobacco seedlings (*N. tabacum* 'Burley

Table 1. Incidence of tobacco mosaic virus (TMV) in tobacco plants after inoculation at topping^a

Treatment	Percentage of plants with TMV based on			
	Mosaic symptoms ^b		Root assay 5 wk after topping ^c	
	At topping	5 wk After topping	ELISA	Plant bioassay ^d
TMV inoculation plus				
Chemical sucker control	0	0	100	100
No chemical sucker control	0	57	100	100
No TMV inoculation plus				
Chemical sucker control	0	0	3	3
No chemical sucker control	0	0	0	0

^aTo inoculate plants, workers' hands were rubbed with ground, TMV-infected tobacco leaves before manually breaking off the tobacco flower stalks (topping).

^bAverage of three replicates of 30 plants each.

^cAverage of three replicates of 10 roots each.

^dTobacco (*Nicotiana tabacum* 'Burley 21') was the indicator plant. Local lesions 5 days after inoculation recorded as positive for TMV.

21') with ground root tissue of the sample being tested. Local lesions were recorded after 5 days. For controls, 10 plants were rubbed with extracts from a ground tobacco leaf known to be infected with TMV. Another 10 plants were rubbed with extracts from a ground tobacco leaf free of TMV.

RESULTS

No visual symptoms of TMV were evident in the field in plants in any treatment before topping. Thirty-three days after topping, mosaic symptoms typical of TMV were observed on 57.3% of the plants in the inoculated plots where no chemical sucker control was applied. No visual symptoms of TMV were noted in any of the other treatments (Table 1).

ELISA and plant bioassay showed TMV in 100% of the roots sampled from plots inoculated with TMV at topping, regardless of whether or not a chemical for sucker control had been applied. There was a low incidence of TMV infection (3% plants infected) in uninoculated plants in plots with chemical sucker control, whereas the plants in the inoculated plot with no sucker control were free of TMV.

DISCUSSION

TMV overwinters in contaminated root debris from infected tobacco plants (6). The transmission of TMV at topping and its subsequent buildup in the roots of infected plants within 5 wk can result in large amounts of debrisborne inoculum for the subsequent crop. Tobacco plants usually remain in the field longer than 5 wk after topping. This aspect of the epidemiology of tobacco mosaic previously has not been confirmed, and its neglect may be one reason losses to TMV remain high despite intensive control programs. Greater emphasis on eradicating debrisborne inoculum by crop rotation and use of TMV-resistant cultivars may be necessary. Also, the prevention of the spread of TMV during topping by washing workers' hands

periodically with hand soap should be tested (4).

TMV was present in the roots of inoculated plants whether sucker growth occurred or was controlled by the use of long-chain aliphatic alcohols and maleic hydrazide, although the chemicals obscured symptoms of TMV infection. This symptomless infection may contribute to the widespread belief among growers that TMV infection tends to abate late in the season.

The low incidence of TMV infection in the uninoculated plot with chemical sucker control apparently resulted from plant-to-plant virus contamination during plot maintenance activities and not from TMV in soilborne crop debris, because tobacco had not been planted in this location for at least 2 yr. Also, in the year before this test, the plot was disked to be maintained as a weedfree fallow.

The results of this study may also have implications in the epidemiology and control of the many strains of TMV that infect tomato. TMV-infected tomato root debris serves as a source of inoculum for subsequent crops (1,5). The ease of mechanical transmission of TMV may result in a proliferation of inoculum during late-season hand labor, such as tying and harvesting tomato crops.

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